INFLUENCES OF SOIL TEXTURE, BIOTA AND FERTILIZERS ON COMMUNITY LEVEL PHYSIOLOGICAL PROFILE

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Abstract. *Nowadays, fertilizers demands in agricultural fields disturb many essential processes among which soil communities' structures and functions are directly affected. At the basis of all processes in soil stands microbial activity. The entire microbial community is considered the food web controller. Several methods can be applied to measure microbial profile in soil related to their activity. Community respiration rates, biomass dynamics and enzyme activity are commonly used technics to assess functional aspects of soil biologic community. Recently CLPP is widely used for assessing microbial community functional diversity. We hypothesized that community level physiological profile could be influenced by fertilizers (mineral and organic), soil biota (earthworms and collembolans) and soil texture (sandy and loamy). In a greenhouse experiment, 60 microcosms were set up in a 2x2x3 experimental design to observe the pattern of microbial community based on their ability to metabolize a wide range of standardized substrates with a modern profiling technique named Microresp™. Correlation analysis showed that the changes in the catabolic profiles of soil microorganisms in both sandy and loamy soil were significant caused by mineral and organic fertilizer applied. In sandy soil earthworms and collembolan presence, together with mineral and organic fertilizers, activates a microbial functional group with the ability to convert α-Ketoglutaric acid. Fructose sugar is representative in catabolic profiles of sandy soil without inoculated biota, independent to the application of fertilizers. Loamy soil treatments promote a microbial biomass, dominant in community structure, capable of metabolizing fructose and malic acid substrate and partly α-Ketoglutaric acid. Based on observed results, application of fertilizers in the presence of soil biota, act to increase the complexity of functional profile. The entire soil communities show a high capacity of turnover due to the application of experimental treatments. Non-rhizospheric experimental setup provides the opportunity to study the overall response of soil community to treatments, even at low values. Mineral fertilizers act toward a reduction of the microbial activity, as an opposite effect visible in organic treated microcosms. Inoculated fauna enhanced a different community level physiological profile, dependent on soil texture - higher in sandy soil. In loamy microcosms it was observed that fertilization produces a powerful constrains on biological community. The most diversified microbial functional profile was observed in soils treated with organic fertilizer.*

Keywords: *collembolans, earthworms, manure, MicroResp, NPK*

INTRODUCTION

Agricultural impacts had led to an increased attention for soil biodiversity losses. If we add an environmental policy, as the renewable resources directive context (DIRECTIVE 2009/28/EC), which requires the extension of energy crops areas and the nowadays need of fertilizers use for obtain high yields, it is supposed that some changes in soil microbial community will appear.

One of the basic approaches regarding soil community is the assessment of functional profiling, which establishes the interactions that are carried out by organisms (NANNIPIERI et al. 2002; WINDING et al. 2005, BASTIDIA et al. 2008). This type of approach can be used as a powerful tool for evaluating soil status (SPEEDING et al. 2004) and the direct associated processes. MicroResp method is a new modern profiling technique to observe the pattern of soil microbial community based on its ability to metabolize a wide range of substrates

(CAMPBELL et al. 2003). The concept of this method is to measure simultaneously the responses of microbial groups to a wide range of carbon based substrates, metabolized separately within an incubation period (BLACK et al. 2011). This data-rich method it has been shown to be discriminatory for the soil type and ecological background, and it is likely to respond quickly to a wide variety of factors as fertilizers use, agricultural technologies and climate changes.

Therefore this experiment aim was to answer some questions about biological activity in soil. One of them was in which way different type of fertilizers influence community level physiological profile? Do earthworms, due to their activity on enhancing nitrogen cycle, soil air/water report and collembolan activity, change microbial physiological profile? What are the main carbon sources metabolized by soil functional community in sandy and loamy soil texture? What kind of functional microbial group is activated during the decomposition of organic dry material of *Silphium perfoliatum*?

MATERIAL AND METHODS

The experiment was set up in microcosms consisting of cylindrical polyvinyl chloride containers each of which was 15 cm high, 19 cm wide, filled with 2 kg sieved (2 mm) soil to a bulk density of 1.2 g cm^{-3}. The soil was taken from the upper 15 cm of two different agricultural fields, was dried on room temperature and defaunated by freezing at -20ºC. First soil properties was represented by sandy texture (58% sand, 20% loam, 22% dust), 6.35 pH, 2.4 humus content, 1.07% organic carbon and 0.09% total nitrogen. Another soil texture was loamy (27% sand, 45% loam, 27% dust) with 6.85 pH, 2.7 humus content, 1.33% organic carbon and 0.11 % total nitrogen. Soil moisture was adjusted and maintained at initial value of 20% humidity the entire experimental trial.

A number of 60 microcosms were set up in the greenhouse (temperature 20ºC, humidity 45%) following a randomized block design (Table 1). To simulate the effects of a perennial energy crop, at the soil surface was spread 6 grams of organic dry material of *Silphium perfoliatum* L. to promote micro-, mezo- and macrofauna activity.

In order to create three differentiated fertilization levels were added 70 g/microcosm of manure, 1.9 g/microcosm of $N_{15}P_{15}K_{15}$ mineral fertilizer together with one treatment with no fertilizer as control.

Each microcosm was inoculated with 2 earthworms (*Lumbricus terrestris* species) and 400 collembolans (*Folsomia candida* species) – considered as soil biota (mix treatment).

Microcosms were closed at the top and bottom with mulch mesh to keep inoculated soil biota for escaping and the experiment last for 7 weeks.

Community level physiological profile was assessed using MicroResp™ method two times: at the start and at the end of experiments. Each soil and substrate combination was replicated two times within each incubation run. The precision of the CLPP assay was assessed by considering the two internal replicates.

For incubations, the soil was supplied with substrates representing: amino acids: L-alanine (L.ala), arginine (Argi), L.cysteine (L.cys), aminobutiric acid (Aminobut), L-lysine (L.lys), N-acetil-glucosamine (N.acetil.gluc); carbohydrates: L-arabinose (L.ara), fructose (Fruct), glucose (Gluc), galactose (Gal), trehalose (Treha); carboxylic acids: citric acid (Citric.ac), malic acid (Malic.ac), oxalic acid (Oxalic.ac), ketoglutaric acid (Keto.ac). For determine basal respiration it was used distilled water (Apa.dist).

All statistical analyses of data were performed using the program Statsoft Statistica 10. For the accomplishment of research aim, Pearson correlation coefficient was calculated for all substrate responses to the presence/absence of experimental factors.

Table 1

Experimental design

Number of experimental treatments	EXPERIMENTAL FACTORS							
	SOIL		PLANT	COMMUNITY (NUMBER OF INDIVIDUALS)		FERTILIZERS		
	Sandy (1)	Loamy (2)	*Silphium perfoliatum*	Mix (1)	Control (2)	GG (1)	IM (2)	FI (3)
M1	+	-	+	+(402)	-	+	-	-
M2				+(402)	-	-	+	-
M3				+(402)	-	-	-	+
M4	+	-	+	-	+(-)	+	-	-
M5				-	+(-)	-	+	-
M6				-	+(-)	-	-	+
M7	-	+	+	+(402)	-	+	-	-
M8				+(402)	-	-	+	-
M9				+(402)	-	-	-	+
M10	-	+	+	-	+(-)	+	-	-
M11				-	+(-)	-	+	-
M12				-	+(-)	-	-	+

GG=organic fertilizer - manure,
IM=mineral fertilizer - $N_{15}P_{15}K_{15}$,
FI=without fertilizers

RESULTS AND DISCUSSIONS

At the beginning of the experiment (Fig. 1) community level physiological profile in sandy soil was characterized by a dominant functional group that decomposes α-ketoglutaric acid and citric acid.

Same results were found in a rhizosphere sandy soil experiment (MIMMO et al. 2008). In loamy soil we can distinguish increased values of arginine decomposition and also, a higher basal respiration rates.

Sandy soil and *S. perfoliatum* biomass stimulates a limited number of functional microbial groups, specialized in metabolizing: α-ketoglutaric acid, fructose, citric and malic acids (Fig. 2).

These groups exceed the 2 mg CO_2-C/g/h due to the synergic influence of fertilization and presence/absence of the biological community.

Both biota presences, stimulates α-ketoglutaric acid decomposition, but only under the conditions provided by fertilizers, and citric acid decomposition, with CO_2 values between 2-3 mg C/g/h (ROMANIUK et al. 2011). The absence of the biological community stimulates the decomposition of fructose, with large variations in fertilized microcosms.

Arginine is decomposed by microbial community mainly in the microcosms without fertilization and only in the absence of biota. Citric acid is not metabolized in the absence of fertilizers and biological community.

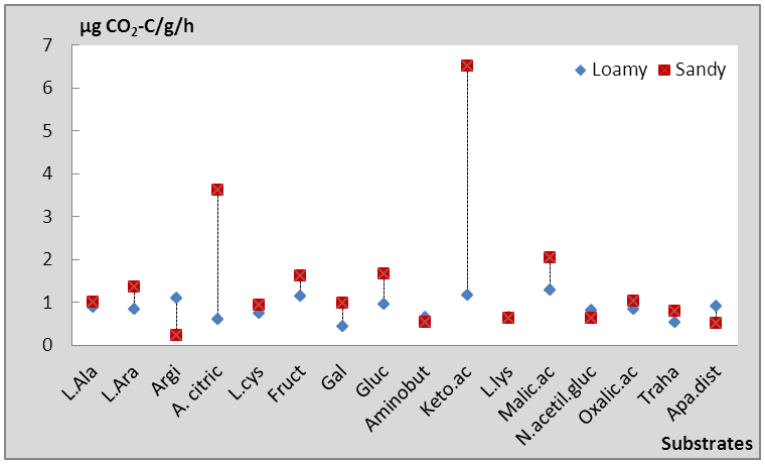

Fig. 1 Community level physiological profile at the beginning of the experiment from microcosms with loamy and sandy soil

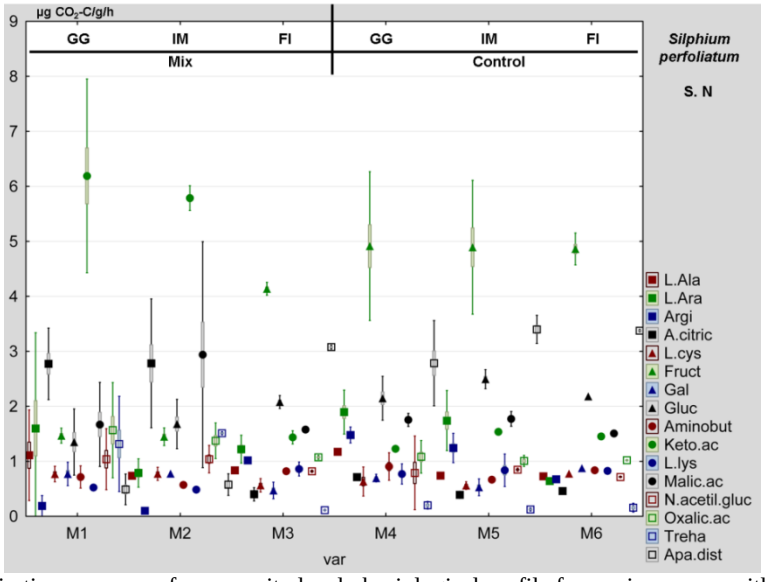

Fig. 2 Respiration response of community level physiological profile from microcosms with sandy soil and *Silphium perfoliatum* organic dry material (GG=manure, IM= mineral fertilizer, FI= without fertilizer, Mix= *L. terrestris*+ *F. candida*, Control= no biota inoculated)

However glucose is more strongly metabolized in the absence of the biological community. Oxalic acid is a substrate which is maintained at substantially the same values, independently to the experimental variables. A similar reaction is visible for L-cysteine, galactose, L-lysine and aminobutyric acid.

The entire experiment, basal respiration values are higher in the treatments without inoculated biological community, which proves a higher potential microbial activity.

Loamy soil reduces microbial activity, regardless of fertilization and biological community (Fig. 3).

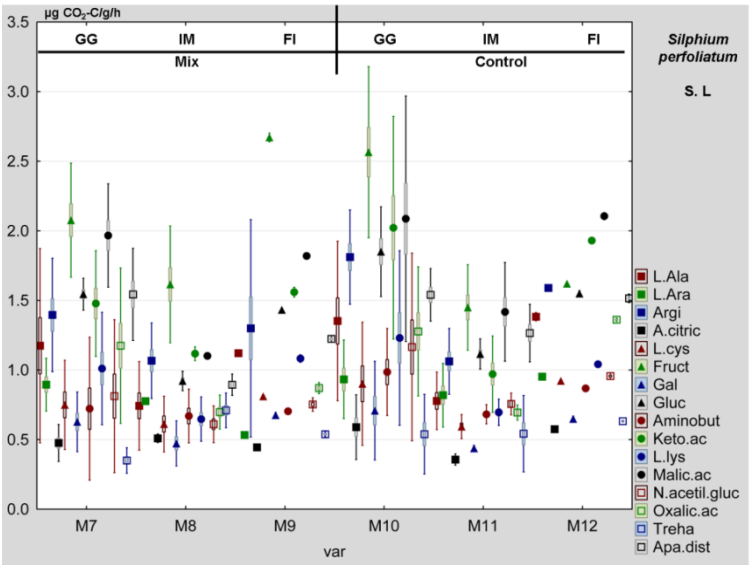

Fig. 3 Respiration response of community level physiological profile from microcosms with loamy soil and *Silphium perfoliatum* organic dry material (GG= manure, IM= mineral fertilizer, FI= without fertilizer, Mix= *L. terrestris*+ *F. candida*, Control= no biota inoculated)

Mineral fertilizer produces lower substrates utilization. Malic acid is better decomposed in the microcosms with organic fertilizer and in absence of fertilization, with higher values in the fauna absence. This phenomenon is visible also to arginine, glucose and oxalic acid. Manure, stimulates the breakdown of fructose and α-ketoglutaric acid, with higher values in the absence of biological community. Citric acid, aminobutyric acid, L cysteine and galactose substrates are metabolized at the same values, regardless of experimental variables which make them good indicators of disturbances. L. alanine and L lysine exceed the 1 mg CO_2-C/g/h only in unfertilized treatment and when manure is used as fertilizer independently of the soil inoculated biota. Similar results ranges were reported in other studies (CREAMER et al. 2016, ROMANIUK et al. 2011).

In general, basal respiration indicates that overall activity of microbial community is higher when manure is applied compared to mineral fertilization. Lower values were observed in the unfertilized treatments with no soil fauna.

Organic dry material of *S. perfoliatum* increase both microbial activity and the breakdown of these substrates.

Soil texture and soil biological community inoculated substantially arginine breakdown in conditions of sandy soil and mixed community, but increases the decomposition of citric acid. Loamy soil, acts to stimulate the decomposition of arginine in the absence of the biological community, but reduces decomposition of citric acid (Table 2).

Fertilization acts at achieving heterogeneous effects on the activity of sugars and amino acids (Table 2).

The only substrates which correlates positively with organic fertilization is L. alanine (r= 0.5), galactose (r= 0.3), aminobutyric acid (r= 0.4), N.acetil.gluc (r= 0.4) and oxalic acid (r= 0.3). Non-application of fertilization leads to decomposition of L. cysteine (r= 0.2) and L. lysine (r= 0.3) and mineral fertilization acts for restricting aminobutyric acid (r= -0.6), L.

alanine (r= -0.5), L. cysteine (r= -0.5), galactose (r= -0.5), L. lysine (r= -0.5), oxalic acid (r= -0.5), arginine (r= -0.3), glucose (r= -0.3) and N.acetil.gluc (r= -0.3).

Pearson correlations emphasize that there are differences between organic carbon substrates based of soil texture as factor. Therefore sandy soil is negatively correlated with almost all tested substrates except trehalose (r= 0.3) and loamy soil indirectly correlated compared to sandy soil. Inoculated soil community have very significantly negative influence of arginine decomposition (r= -0.3) and positive effect of citric acid, α-ketoglutaric acid and trehalose (r= 0.3).

Table 2

Pearson correlation between substrate decomposition and presence/absence of experimental factors

	Textura solului Soil texture		**Comunitatea Community**		**Fertilizantul Fertilizer**		
	Sandy	***Loamy***	***Mix***	***Control***	***GG***	***IM***	***FI***
L.ala	0.1	-0.1	0.0	0.0	0.5***	-0.5***	0.0
L.ara	-0.7***	0.7***	0.0	0.0	0.2	-0.1	-0.1
Argi	0.1	-0.1	-0.3***	0.3***	0.1	-0.3***	0.1
Citric.ac	-0.3***	0.3***	0.3***	-0.3***	0.2	0.0	-0.2
L.cys	0.2	-0.2	0.0	0.0	0.2	-0.5***	0.2***
Fruct	-0.7***	0.7***	-0.1	0.1	0.1	-0.1	0.1
Gal	-0.4***	0.4***	0.0	0.0	0.3***	-0.5***	0.2
Gluc	-0.7***	0.7***	-0.1	0.1	0.2	-0.3***	0.2
Aminobut	-0.2	0.2	-0.2	0.2	0.4***	-0.6***	0.2
Keto.ac	-0.4***	0.4***	0.3***	-0.3***	0.2	0.0	-0.2
L.lys	0.0	0.0	-0.2	0.2	0.2	-0.5***	0.3***
Malic.ac	-0.3***	0.3***	0.2	-0.2	0.2	-0.2	0.0
N.acetil.gluc	-0.3***	0.3***	0.0	0.0	0.4***	-0.3***	-0.1
Oxalic.ac	-0.4***	0.4***	0.1	-0.1	0.3***	-0.5***	0.2
Treha	0.3***	-0.3***	0.3***	-0.3***	0.0	0.2	-0.2
Apa.dist	-0.7***	0.7***	-0.1	0.1	-0.1	-0.1	0.1

p<0.05*, p<0.01**, p<0.001***

For abbreviations see Figure 2 and Figure 3 (GG=manure, IM=mineral fertilizer, FI=without fertilizer)

CONCLUSIONS

These detailed analyses results appear to increase the complexity of soil microbial biomass functional profile when fertilizers and other biota organisms are present and makes the interpretations more difficult.

One primary reason for this gap is microbial overwhelming diversity and a lack of knowledge about how this diversity is related to ecosystem processes.

A reduction of microbial activity was observed in the microcosms where mineral fertilizers were added as an opposite effect was in organic treatments.

Respiration responses were higher in sandy soil and it was enhanced by inoculated fauna.

In microcosms with loamy soil it was observed that fertilization produces a reduction of biological community. The most diversified microbial functional profile was observed in soils with organic fertilizer. The main sources of metabolized carbon in sandy soil were α-ketoglutaric acid and citric acid substrates and in loamy soil fructose and malic acid respectively. Addition of *S. perfoliatum* dry material cause catabolic response of all substrates and showed differences among fertilizers management and soil texture.

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